PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 15010pc1	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2004/000488	International filing date *(day/month/year)* 07.07.2004	Priority date *(day/month/year)* 09.07.2003

International Patent Classification (IPC) or national classification and IPC
A61K39/39, A61K39/04, A61P31/06

Applicant
STATENS SERUM INSTITUT

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03.02.2005	18.01.2006

Name and mailing address of the international preliminary examining authority:
European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer
Teyssier, B
Telephone No. +31 70 340-2062

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2004/000488

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-37 as originally filed

   Claims, Numbers

   1-11 received on 08.12.2005 with letter of 06.12.2005

   Drawings, Sheets

   1-11 as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 Dascher C et al., *International Immunology* August 2003, 15(8), 915-925

D2 WO 03/011336 A (National Research Council of Canada) 13 February 2003

D3 WO 02/074333 A (Stanford Rook Ltd.) 26 September 2002

D3a WO 02/074330 A (Stanford Rook Ltd.) 26 September 2002

D4 Moura A et al., *Scandinavian Journal of Immunology* November 1997, 46(5), 500-505

D1 discloses a mycobacterial vaccine comprising as antigen mycobacterial lipids extracted with chloroform:methanol 2:1 (therefore presumably total lipids) formulated as liposomes vesicles with cholesterol:DSPC and, possibly, QS-21 saponin adjuvant or the cationic adjuvant DDA (p. 916-917). The conclusion suggests to combine both protein and lipid *antigens* from mycobacteria (p. 924, last §), but such a composition is not illustrated and the *adjuvant* properties of mycobacterial lipids are not shown.

D2 teaches the adjuvant properties of polar lipids from BCG (see extraction procedure p. 9 and 14) formulated as liposomes using neutral surfactants (p. 9-10 and 16-17). D2 fails to provide teachings regarding the suitability of apolar mycobacterial lipids or of total mycobacterial lipids as adjuvants.

D3 and D3a teach the Th1 adjuvant properties of various fractions of mycobacterial lipids, especially fraction 4 (obtained from a methanol layer after separation of a petrol layer, thus presumably polar lipids, see example 1) and its sub-fractions 7 and 8. D3 and D3a teach that fraction 3, produced from a petrol layer and therefore presumably comprising apolar lipids, has no activity (example 2), thus teaching away from the use of apolar lipids as adjuvants. No incentive is given to formulate the mycobacterial lipids with cationic surfactants, which has been shown in the present examples (tables 7-8) as resulting in a Th1 adjuvant activity.

In apparent contradiction with the present teachings, D4 teaches the *inhibitory* effects of liposomes prepared from neutral (apolar) lipids of *Mycobacterium leprae*. However, D4 does not make use of a cationic surfactant; it is thus impossible to conclude whether the results of D4 derive from the lack of a cationic surfactant or from a structural difference in the apolar lipids of *M. leprae*, a species which diverges from the majority of mycobacterias.

In view of this prior art, adjuvants comprising the apolar fraction of mycobacterial lipids and a cationic surfactant are new and an inventive step can be acknowledged because the adjuvant properties of the *combination* of apolar mycobacterial lipids with a cationic surfactant cannot be anticipated. Thus claims 1-11 meet the requirements of Article 33(2-4) PCT.

Claims

1. An adjuvant comprising a cationic surfactant and the apolar fraction or part of the apolar fraction of the total lipid extract of a mycobacterium, e.g. the BCG, *M.microti, M.tuberculosis* and *M.vaccae.*

2. An adjuvant according to claim 1 where the part of the apolar fraction of the lipid extract can be phthiocerol dimycocerosates, trehalose mycolipenates, glycosylated phenol phthiocerols (including phenolic glycolipids, PGL's), trehalose mycolates, sulfolipids, triacylglycerols or menaquinones

3. An adjuvant according to claim 1-2 where the surfactant is DDA, DODA, DC-chol or DOTAP.

4. An adjuvant according to claim 1-2 where the surfactant is neutral or anionic, e.g. DOPE/PC or DOPE/PC/PG.

5. A vaccine comprising an adjuvant according to claim 1-4.

6. A vaccine according to claim 5 for parenterally, oral or mucosal administration.

7. A vaccine according to claim 6 where the antigenic component comprises an antigenic epitope from a virulent mycobacterium, e.g. *Mycobacterium tuberculosis, M. bovis* or *M.africanum.*

8. A vaccine according to claim 7 where the antigenic component is an ESAT6-Ag85B hybrid or a fragment hereof.

9. A vaccine according to claim 6 for treating cancer, allergy or autoimmune diseases.

10. A delivery system comprising an adjuvant according to claim 1-4.

11. Preparing an adjuvant according to claim 1-4 using thin lipid film method.